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(54) Title: INACTIVATION OF GRAM-POSITIVE BACTERIA

(57) Abstract: A method for inactivating medically important Gram-positive bacteria including Methicillin-resistant *Staphylococcus aureus* (MRSA), Coagulase-Negative *Staphylococcus* (CONS), *Streptococcus*, *Enterococcus* and *Clostridium* species, comprising exposure to visible light, and in particular light within the wavelength range 400-500 nm.

INACTIVATION OF GRAM-POSITIVE BACTERIA

The present invention relates to a method for inactivating medically important Gram-positive bacteria including *Staphylococcus aureus* and methicillin (multi)-resistant *Staphylococcus aureus* (MRSA), Coagulase-Negative *Staphylococcus* (CONS), *Streptococcus*, *Enterococcus* and *Clostridium* species.

Background of the Invention

Methicillin-resistant *Staphylococcus aureus* (MRSA) is becoming an increasingly problematic micro-organism, with infection rates rising and effective methods of control becoming more and more limited. In addition to the resistance of MRSA to antibiotics, there is a significant problem due to the availability of few effective sterilisation methods for environmental decontamination; for example in air and on contact surfaces. Public and media interest in the transmission and control of MRSA is escalating and it is becoming one of the most significant problems within the healthcare industry. Hospitals and nursing homes are the worst affected areas. Furthermore, community-acquired MRSA is also now being recognised as an increasing problem, with transmission occurring in public and social areas such as public gyms and sports centres.

As well as MRSA, other Gram-positive bacteria are known to cause health problems, particularly in the hospital environment. For example, *Staphylococcus epidermidis*, which is a Coagulase-Negative *Staphylococcus* (CONS), can cause infection, particularly in infants and in hospitalised patients who have received prosthetic implant surgery. *Streptococcus pyogenes* is a Gram-positive coccus commonly associated with infections such as pharyngitis, pyoderma, scarlet fever, erysipelas, cellulitis, streptococcal toxic-shock syndrome, rheumatic fever, glomerulonephritis, bacteraemia and necrotizing fasciitis, often referred to as "flesh-eating bacteria". *Enterococcus faecalis* (another Gram-positive coccus) is a common cause of urinary tract and wound infections, as well as other infections including bacteraemia, endocarditis and meningitis in severely ill hospitalised patients. Multi-antibiotic resistance is also becoming a well-documented problem with enterococcal infections. *Clostridium* species, in particular *C. difficile*, have been associated with high mortality in elderly patients due to diarrohea-associated dehydration, medically known as antibiotic-associated pseudomembranous colitis.

Many techniques have been proposed for destroying harmful bacteria, such as MRSA. For example, US 6,251,127 describes a photodynamic process for the inactivation of bacteria and fungal wound infections using methylene blue or toluidene blue. Light energy in combination with photosensitising agents is used to treat or detect pathologies of living tissue, including cancer and microbiological pathogens. The light used has wavelengths ranging from about 450 nm to about 850 nm. Tests demonstrate the efficacy of the light treatment in combination with the photosensitising agents for the destruction of *Staphylococcus aureus* in *in-vivo* infected wounds; and for *in-vitro* destruction of antibiotic-resistant *Staphylococcus*, *Streptococcus*, *Enterococcus*, *E. coli*, *Pseudomonas*, *Haemophilus influenza* and *Candida albicans*. In addition, wavelength spectra of activation of methylene blue and toluidene blue in the presence of various concentrations of the above bacteria and *Candida* have been provided.

Whilst in some environments, the methodology of US 6,251,127 may be useful, it nevertheless suffers from the significant practical disadvantage that photosensitising agents must be applied to the bacteria that are to be inactivated. A similar problem arises with US2005/0049228, which also requires the combined use of a photosensitiser and light; in this case, in the range of 500 nm to 580 nm. The need for photosensitising agents is a significant limitation of these techniques.

An objective of the present invention is to provide a simple and effective technique for inactivating selected bacteria, in particular MRSA, and more generally the *Staphylococcus, Streptococcus, Enterococcus* and *Clostridium* species.

Summary of the Invention

A method for inactivating one or more pathogenic gram-positive bacterial comprising exposure of the bacteria to visible light without using a photosensitiser.

Preferably said bacteria are selected from *Staphylococcus*, in particular MRSA, CONS, *Streptococcus*, *Enterococcus* and *Clostridium* species.

It is understood that the term pathogenic is used in the context of gram-positive bacterial species and/or strains, which are capable of causing disease or infection in a human or

animal subject. It is also understood that some bacteria are often commensal in that they are able to colonise and/or live on/within a healthy host and not become pathogenic unless or until the host becomes immunocompromised and/or unhealthy due to some other form of disease or injury, such as a wound. Such "potentially" pathogenic bacteria are encompassed by the invention also.

Moreover, the term inactivation is understood to mean that said bacteria are killed, or damaged so as to reduce or inhibit bacterial replication. The methods and systems taught herein can therefore be considered as bactericidal and/or bacteriostatic and this may depend on the species/strain of bacteria, wavelength of light, dose, etc.

Exposing these bacteria to blue light, or white light containing blue light, has been found to stimulate an inactivation process. An advantage of using light in the visible-wavelength region is that there is no detrimental effect on human or animal health. Consequently, the method can be used for an extensive range of applications, such as air disinfection, contact-surface and materials disinfection and, most noteworthy, wound protection and tissue disinfection.

According to another aspect of the invention, there is provided a method for inactivating pathogenic gram positive bacteria including at least one of Methicillin-resistant *Staphylococcus aureus* (MRSA), Coagulase-Negative *Staphylococcus* (CONS), *Streptococcus*, *Enterococcus* and *Clostridium* species comprising exposure of the bacteria to visible light having a wavelength in the range 400-500 nm. The visible light may have a wavelength in the range 400-450 nm. The light may have a wavelength in the range 400-420 nm. The light may have a wavelength of 405 nm.

According to yet another aspect of the invention, there is provided a system for inactivating pathogenic Gram-positive bacteria including Methicillin-resistant *Staphylococcus aureus* (MRSA), Coagulase-Negative *Staphylococcus* (CONS), *Streptococcus*, *Enterococcus* and *Clostridium* species, comprising the means for exposing them to visible light having a wavelength in the range of 400-500 nm. The wavelength of the light used is preferably in the range 400-500 nm. The wavelength may be in the range 400-450 nm, and more specifically in the range 400-420 nm, with optimal inactivation at 405 nm.

According to still another aspect of the invention, there is provided use of visible light having a wavelength in the range of 400-500 nm, especially 400-420 nm for inactivating pathogenic gram positive bacteria including at least one of Methicillin-resistant *Staphylococcus aureus* (MRSA), Coagulase-Negative *Staphylococcus* (CONS), *Streptococcus*, *Enterococcus* and *Clostridium* species.

Brief Description of the Drawings

Various aspects of the present invention will now be described by way of example only and with reference to the accompanying drawings, of which:

- Figure 1 shows the total emission spectrum of a Hamamatsu Xenon lamp;
- Figure 2 shows in greater detail the ultra-violet emission spectrum of the Xenon lamp of Figure 1;
- Figure 3 is a plot of bacterial count of a methicillin-resistant *S. aureus* strain as a function of time of exposure to light of wavelength greater than 400 nm;
- Figure 4 is a plot of bacterial count of a second methicillin-resistant *S. aureus* strain as a function of time of exposure to light of wavelength greater than 400 nm;
- Figure 5 is a plot of bacterial count of *S. aureus* NCTC 4135 as a function of time of exposure to light of wavelength greater than 400 nm;
- Figure 6 is a plot of bacterial count of *S. epidermidis* NCTC 7944 as a function of time of exposure to light of wavelength greater than 400 nm;
- Figure 7 is a plot of bacterial count of *Streptococcus pyogenes* NCTC 8198 as a function of time of exposure to light of wavelength greater than 400 nm;
- Figure 8 is a plot of bacterial count of *Enterococcus faecalis* as a function of time of exposure to light of wavelength greater than 400 nm;
- Figure 9 is plots of bacterial count in a suspension of *S. aureus* NCTC 4135 as a function of time of exposure to light for different wavelength ranges;
- Figure 10 is a plot of bacterial log reduction as a function of wavelength (400-500 nm) for *S. aureus* NCTC 4135;
- Figure 11 is plots of bacterial count in a suspension of *S. aureus* NCTC 4135 as a function of time of exposure to light of wavelength greater than 400 nm for different light intensities;

- Figure 12 is a visual indication of the surface inactivation of *S. aureus* NCTC 4135 through exposure to light of wavelengths greater than 400 nm. Surface inactivation is evidenced by inhibition of *S. aureus* growth on the areas exposed to this light;
- Figure 13 is a plot of bacterial count of *S. aureus* NCTC 4135 as a function of time of exposure to light of 405 nm;
- Figure 14 is a plot of bacterial count of a methicillin-resistant *S. aureus* strain as a function of time of exposure to light of 405 nm;
- Figure 15 is a plot of bacterial count of *Streptococcus pyogenes* NCTC 8198 as a function of time of exposure to light of 405 nm, and
- Figure 16 is a plot of bacterial count of *Clostridium perfringens* 13124 as a function of time of exposure to light of 405 nm.

Detailed Description of the Drawings

Exposing MRSA to blue light has been found to cause significant inactivation. This narrow range of wavelength is part of the white-light spectrum. For all white-light sources, only a small fraction of the light output is in this range, typically one or two percent. Hence, to provide a sufficient amount of light and demonstrate the effectiveness of this technique, the source used was a Xenon lamp (Hamamatsu Photonics UK Limited). Emission spectra of the lamp are shown in Figures 1 and 2. The lamp was used in combination with an optical-fibre light guide and a selection of optical filters in order to allow exposure of the *Staphylococcus aureus* suspensions to specified wavelengths of visible light. The output of the light guide was maintained at a distance of 5 cm from the sample during all experiments.

To demonstrate the effectiveness of the technique, various studies have been carried out. The bacteria used were as follows: *Staphylococcus aureus* NCTC 4135; methicillin-resistant *Staphylococcus aureus* LMG 15975; methicillin-resistant *Staphylococcus aureus* 16a (clinical isolate), *Staphylococcus epidermidis* NCTC 7944, *Streptococcus pyogenes* NCTC 8198 *Enterococcus faecali* and *Clostridium perfringens 13124*. Each sample was serially diluted to the appropriate concentration using phosphate-buffered saline (PBS), plated out using nutrient agar (NA) and then incubated at 37°C for 24 hours.

Suspensions of methicillin-resistant *Staphylococcus aureus* LMG 15975 and clinical isolate 16a were prepared and exposed to visible light. The light was transmitted through a 400 nm long-wave pass filter (50% cut-off in transmission at 400 nm) before impacting on the bacterial suspension. This allowed only wavelengths of 400 nm and above (visible light) to illuminate the sample. The results of these experiments are shown in Figures 3 and 4. From these, it can be seen that the light treatment causes significant reduction in the counts of both the culture collection MRSA (LMG 15975) and the highly resistant clinical isolate (16a). The control data refer to samples that were untreated over the same time interval.

Suspensions of *Staphylococcus aureus* NCTC 4135 were also exposed to visible-light treatment. Again, the light beam was transmitted through a 400 nm long-wave pass filter before impacting on the bacterial suspension, allowing only the transmission of wavelengths of 400 nm and above. From Figure 5 it can be seen that the Xenon light source caused significant reduction in the *Staphylococcus aureus* count even with a high starting bacterial population of approximately 10^7 colony-forming units per millilitre (cfu/ml). Similar experiments were carried out using *Staphylococcus epidermidis* NCTC 7944, *Streptococcus pyogenes* NCTC 8198 and *Enterococcus faecalis*. The associated reductions in the bacterial population are shown in Figures 6, 7 and 8. In each of these a significant reduction in the bacterial count is observed.

Exposure tests using a range of filters were carried out. Bacterial suspensions were exposed to the following wavelength ranges for times up to 90 minutes: greater than 550 nm (using a 550 nm long-wave pass filter); greater than 500 nm (using a 500 nm long-wave pass filter), less than 500 nm (using a 500 nm short-wave pass filter); 400-500 nm (using a 400 nm long-wave pass filter and a 500 nm short-wave pass filter in combination); 450-500 nm (using a 450 nm long-wave pass filter and a 500 nm short-wave pass filter in combination); greater than 450 nm (using a 450 nm long-wave pass filter), and greater than 400 nm (using a 400 nm long-wave pass filter). The resultant inactivation curves in Figure 9 allow only qualitative comparisons to be made since the filters do not have sharp cut-off wavelengths and the light intensities falling on the suspensions were different for the different curves. The results do however indicate that the wavelength region between 400 nm and 500 nm does provide a high rate of *S. aureus* inactivation.

Experiments were also carried out using bandpass filters each with a 10 nm FWHM (full-width, half-maximum). Suspensions of methicillin-resistant *S. aureus* LMG 15975 (approximately 10^5 cfu/ml population) were exposed to visible light transmitted through the following bandpass filters: 400 nm, 405 nm, 410 nm, 415 nm, 420 nm, 430 nm, 440 nm, and 450 nm. The intensity of the lamp was altered for each filter to ensure that the light power at the suspension was the same for each measurement, thus allowing direct comparison of results. The results of these experiments showed that samples exposed using the 400 nm, 405 nm and 415 nm bandpass filters have a reduced colony-forming-unit count/ml; that is, light of wavelengths within these narrow bandwidths had an inactivating effect on the *S. aureus* strains.

A more detailed analysis of wavelength sensitivity was performed using suspensions of *S. aureus* NCTC 4135, and this is shown in Figure 10. The results show that samples exposed using the 400 nm, 405 nm, 410 nm, 415 nm and 420 nm bandpass filters have a reduced colony-forming-unit count/ml; that is, light of wavelengths within these narrow bandwidths had an inactivating effect on the *S. aureus* strains. From these results it can be deduced that visible-light exposure over the wavelength range 400-450 nm is the major inducing factor for Staphylococcal inactivation, with increased inactivation occurring over the range 400-420 nm and optimum inactivation occurring at 405 nm. Moreover, it has been observed that a lower dose is required at this wavelength and typically the dose is less than 200 J/cm^2, such as less than 100 J/cm^2.

In further experiments, *Staphylococcus aureus* NCTC 4135 suspensions were exposed to different intensities of visible-light treatment. These measurements were made using the 400 nm long-wave pass filter, that is, for wavelengths greater than 400 nm. Figure 11 shows the results of these experiments. It can be seen that as the intensity of the light decreases, so to does the inactivation rate. The specific doses required for complete inactivation of Staphylococcal, Streptococcal and Enterococcal species using different filters and light intensities were determined. Sample results are shown in the Table below:

ORGANISM	WAVELENGTH RANGE	DOSE (J/cm^2)	J/cm^2/log reduction
S. aureus 4135	>400nm (100% intensity)	630	126
S. aureus 4135	>400nm (75% intensity)	729	145.8
S. aureus 4135	>400nm (50% intensity)	648	144
S .aureus 4135	<500nm	189.6	37.92
S. aureus 4135	400-500nm	290.8	58.2
MRSA 15975	>400nm	1260	252
MRSA 16a	>400nm	945	189
S. epidermidis NCTC 7944	>400nm	840	168
Strep. pyogenes NCTC 8198	>400nm	1440	288
E. faecalis	>400nm	2880	1440

The effect of visible-light exposure for surface decontamination was also examined. This was done by exposing *S. aureus* cells, which were plated onto nutrient agar, to the light treatment (through a 400 nm long-wave pass filter) prior to incubation. Examples of results are shown as the areas of growth inhibition on the culture plates in Figure 12.

A similar treatment system to that used with the Xenon lamp was assembled using a 405 nm LED array as a light source. Experiments were carried out using *Staphylococcus aureus* NCTC 4135, MRSA 16a, *Streptococcus pyogenes* NCTC 8198 and *Clostridium perfringens* 13124. The associated reductions in the bacterial population are shown in Figures 13, 14, 15 and 16, respectively. The specific doses required for complete inactivation of *Staphylococcus*, *Streptococcus* and *Clostridium* species using the 405 nm LED array were determined. Sample results are shown in the Table below:

ORGANISM	WAVELENGTH	DOSE (J/cm^2)	J/cm^2/log reduction
S. aureus 4135	405nm	36	7.2
MRSA 16a	405nm	45	9
Streptococcus pyogenes NCTC 8198	405nm	54	10.8
Clostridium perfringens 13124	405nm	45	10.2

A comparison of the doses required for bacterial inactivation (5-log reduction) using light of wavelengths greater than 400 nm from the Xenon lamp and a 405 nm LED array is shown in the Table below:

ORGANISM	**DOSE (J/cm^2)**		**J/cm^2/log reduction**	
	>400nm	**405nm**	**>400nm**	**405nm**
S. aureus NCTC 4135	630	36	126	7.2
MRSA 16a	945	45	189	9
Cl. perfringens 13124	1440	54	288	10.8

The use of 400-500 nm, in particular 400-450 nm, wavelengths of visible light (blue light) has proved to be an effective means of inactivation of *Staphylococcus* strains, including MRSA, as well as CONS, *Streptococcus*, *Enterococcus* and *Clostridium*, with increased inhibition rates in the 400-420 nm range and in particular, around 405 nm. This demonstrates that a light source (continuous source, flashlamp, laser etc.) with output at wavelengths in these regions could potentially be used in clinical environments for the reduction in levels of methicillin-resistant *Staphylococcus aureus,* and other medically important Gram-positive species, present in the air and on contact surfaces and materials, and most importantly, could be used for wound protection and tissue treatment. The exact parameters required would depend on the bacterial strain, the wavelength of the light being used and the light intensity. These can be readily determined experimentally.

Variations of the disclosed arrangements are possible without departing from the invention. For example, although both a Xenon lamp with a variety of different filters and a 405 nm LED array have been used as the inactivation source, it will be appreciated that any suitable light source can be used. Equally, although a particular experimental arrangement has been described here, it will be readily apparent that the light source used could be included in, for example, a hand-held device or could be designed to operate in or around areas that have to be kept free of MRSA. Accordingly the above description of the specific embodiment is made by way of example only and not for the purposes of limitation. It is clear that minor modifications may be made without significant changes to the operation described.

Claims

1. A method for inactivating one or more pathogenic gram-positive bacteria comprising exposure of the bacteria to visible light without using a photosensitiser.

2. The method according to claim 1 wherein the bacteria are selected from the group consisting of Methicillin-resistant *Staphylococcus aureus* (MRSA), Coagulase-Negative *Staphylococcus* (CONS), *Streptococcus*, *Enterococcus* and *Clostridium* species.

3. A method as claimed in claim 1 or 2 wherein the visible light has a wavelength in the range 400-500 nm.

4. A method as claimed in claim 3 wherein the visible light has a wavelength in the range 400-450 nm.

5. A method as claimed in claim 4 wherein the light has a wavelength in the range 400-420 nm.

6. A method as claimed in claim 5 wherein the light is in the region of wavelength 405 nm.

7. A method for inactivating one or more pathogenic gram-positive bacteria comprising exposure of the bacteria to visible light having a wavelength in the range of 400-500 nm.

8. The method according to claim 7 wherein the bacteria are selected from the group consisting of Methicillin-resistant *Staphylococcus aureus* (MRSA), Coagulase-Negative *Staphylococcus* (CONS), *Streptococcus*, *Enterococcus* and *Clostridium* species.

9. A method as claimed in claims 7 or 8 wherein the visible light has a wavelength in the range 400-450 nm.

10. A method as claimed in claim 9 wherein the light has a wavelength in the range 400-420 nm.

11. A method as claimed in claim 10 wherein the light is in the region of wavelength 405 nm.

12. A system for inactivating one ore more pathogenic gram-positive bacteria comprising means for exposing said bacteria to visible light having a wavelength in the range of 400-500 nm.

13. The method according to claim 12 wherein the bacteria are selected from the group consisting of Gram-positive bacteria, including Methicillin-resistant *Staphylococcus aureus* (MRSA), Coagulase-Negative *Staphylococcus* (CONS), *Streptococcus*, *Enterococcus* and *Clostridium* species.

14. Use of visible light having a wavelength in the range of 400-500 nm for inactivating one or more pathogenic gram positive bacteria.

15. The method according to claim 14 wherein the bacteria are selected from the group consisting of Methicillin-resistant *Staphylococcus aureus* (MRSA), Coagulase-Negative *Staphylococcus* (CONS), *Streptococcus*, *Enterococcus* and *Clostridium* species.

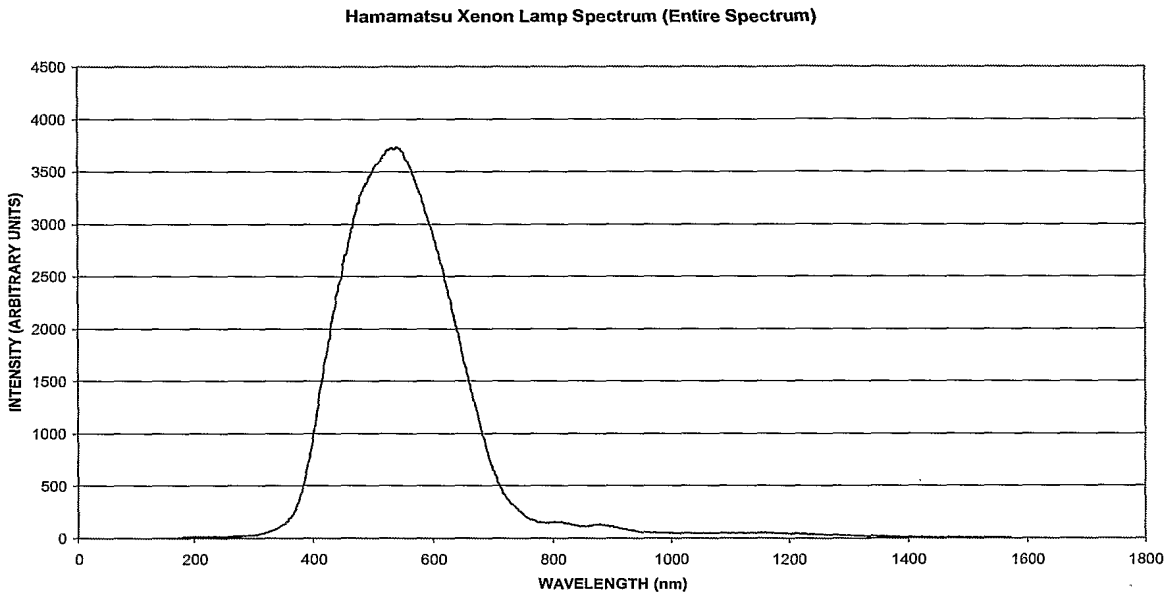
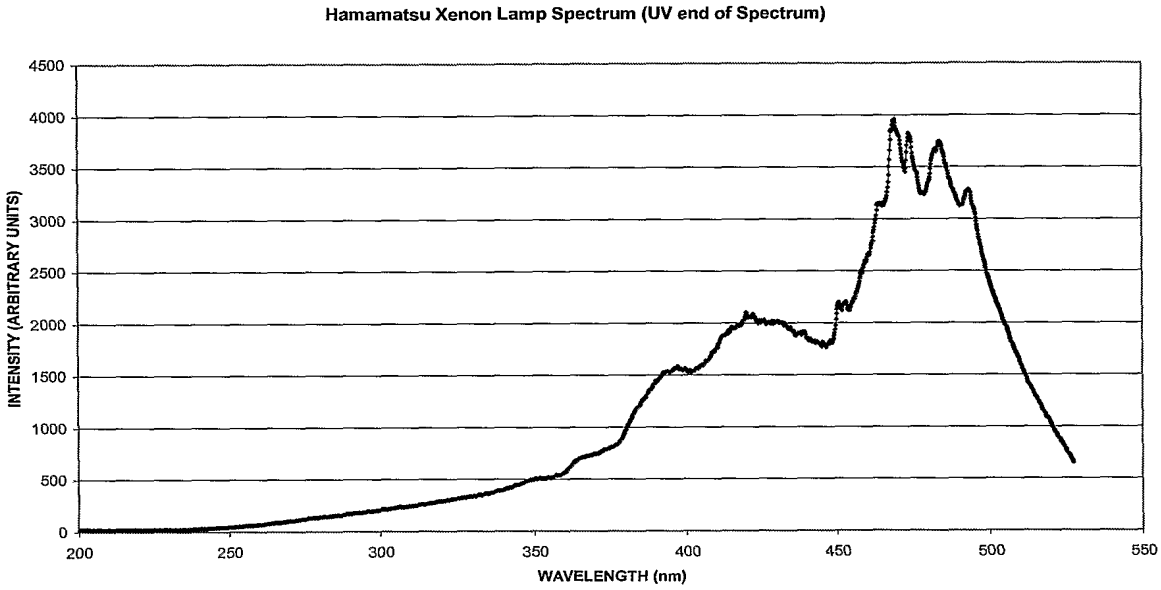

Hamamatsu Xenon Lamp Spectrum (Entire Spectrum)
INTENSITY (ARBITRARY UNITS)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
200
400
600
800
1000
1200
1400
1600
1800
WAVELENGTH (nm)

FIGURE 1


Hamamatsu Xenon Lamp Spectrum (UV end of Spectrum)
INTENSITY (ARBITRARY UNITS)
4500
4000
3500
3000
2500
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1000
500
0
200
250
300
350
400
450
500
550
WAVELENGTH (nm)

FIGURE 2

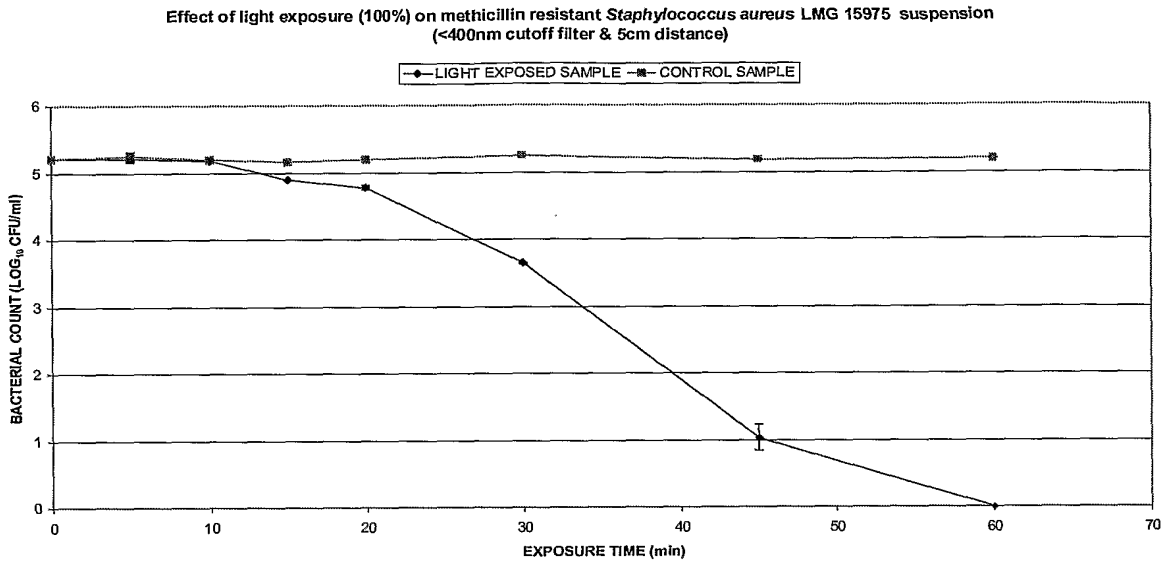
Effect of light exposure (100%) on methicillin resistant Staphylococcus aureus LMG 15975 suspension
(<400nm cutoff filter & 5cm distance)
LIGHT EXPOSED SAMPLE
CONTROL SAMPLE
BACTERIAL COUNT (LOG10 CFU/ml)
EXPOSURE TIME (min)
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70

FIGURE 3

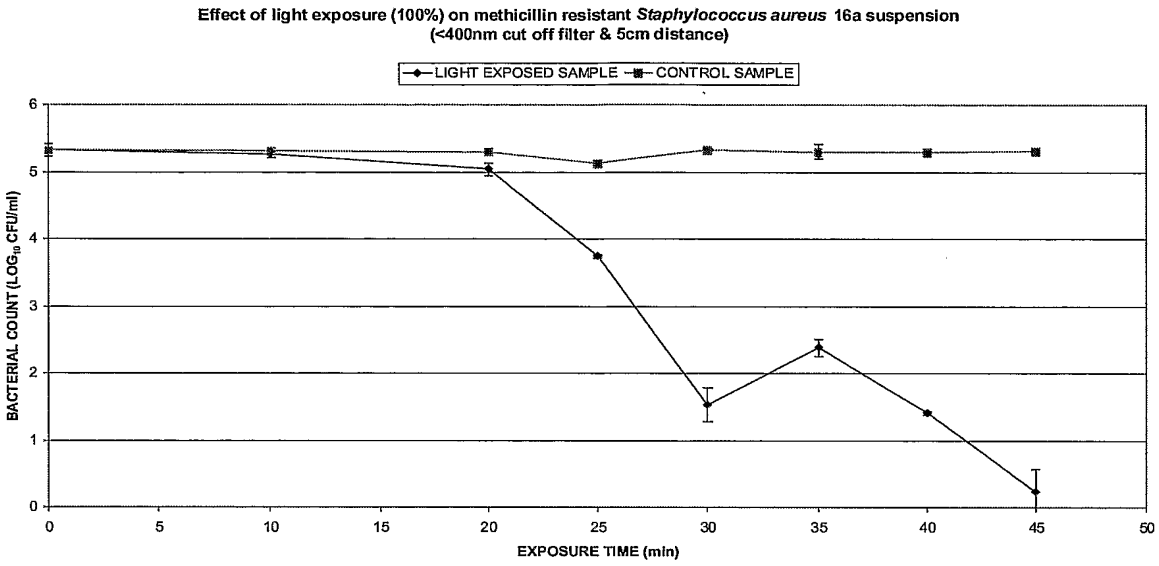
Effect of light exposure (100%) on methicillin resistant Staphylococcus aureus 16a suspension
(<400nm cut off filter & 5cm distance)
LIGHT EXPOSED SAMPLE
CONTROL SAMPLE
BACTERIAL COUNT (LOG10 CFU/ml)
EXPOSURE TIME (min)
0
1
2
3
4
5
6
0
5
10
15
20
25
30
35
40
45
50

FIGURE 4

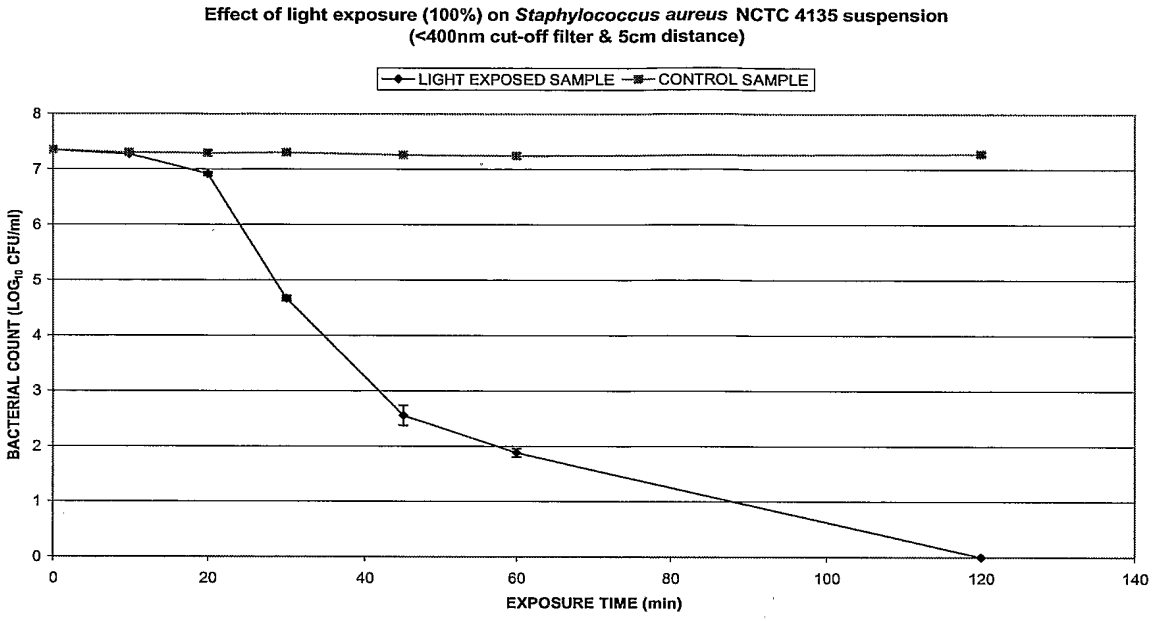
Effect of light exposure (100%) on Staphylococcus aureus NCTC 4135 suspension
(<400nm cut-off filter & 5cm distance)
LIGHT EXPOSED SAMPLE
CONTROL SAMPLE
BACTERIAL COUNT (LOG10 CFU/ml)
EXPOSURE TIME (min)
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100
120
140

FIGURE 5

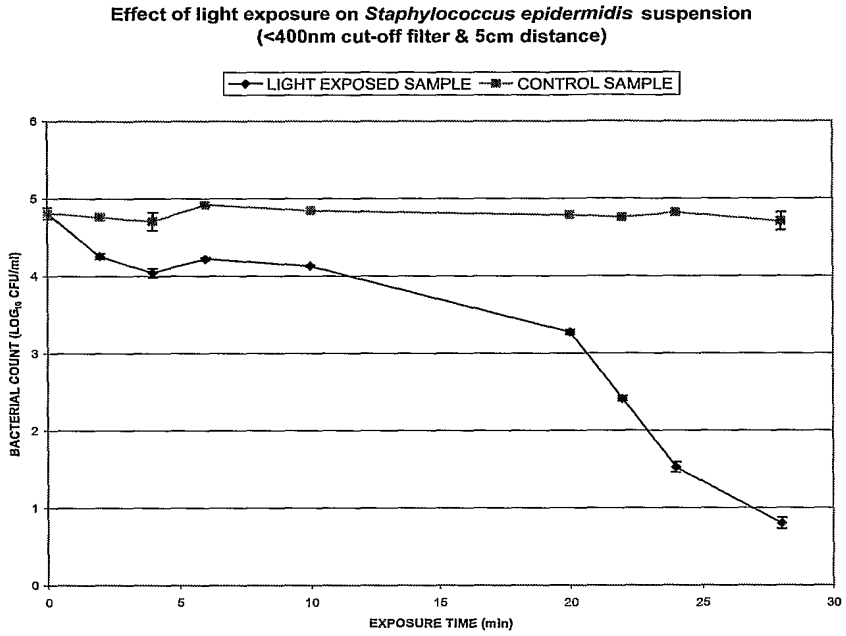
Effect of light exposure on *Staphylococcus epidermidis* suspension
(<400nm cut-off filter & 5cm distance)
LIGHT EXPOSED SAMPLE
CONTROL SAMPLE
BACTERIAL COUNT (LOG10 CFU/ml)
EXPOSURE TIME (min)
0
1
2
3
4
5
6
0
5
10
15
20
25
30

FIGURE 6

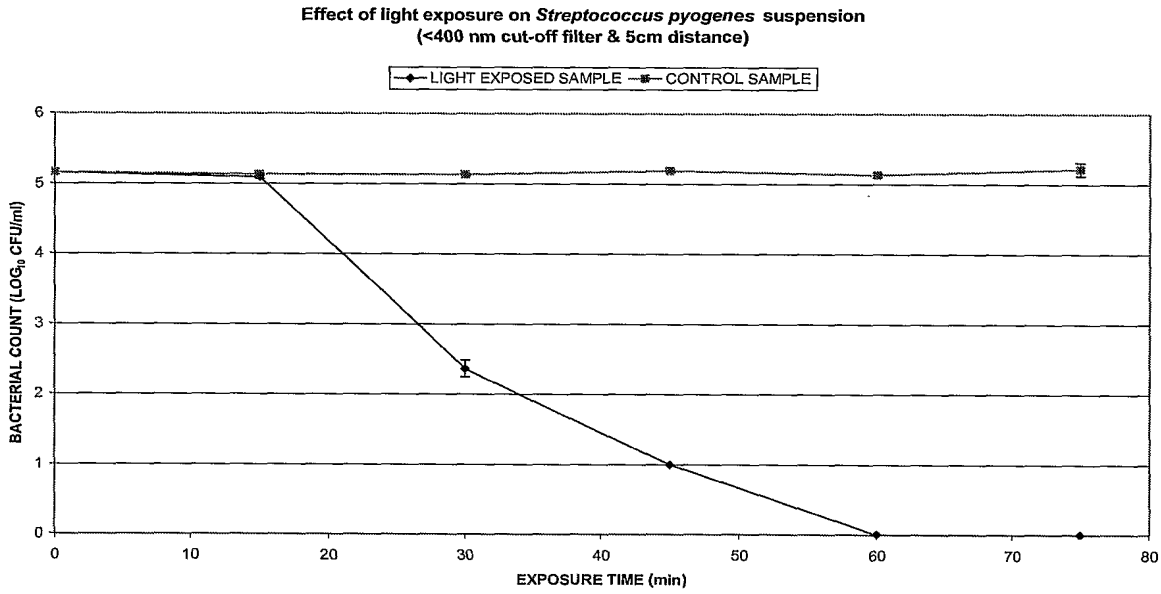
Effect of light exposure on *Streptococcus pyogenes* suspension
(<400 nm cut-off filter & 5cm distance)
LIGHT EXPOSED SAMPLE
CONTROL SAMPLE
BACTERIAL COUNT (LOG_{10} CFU/ml)
EXPOSURE TIME (min)
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70
80

FIGURE 7

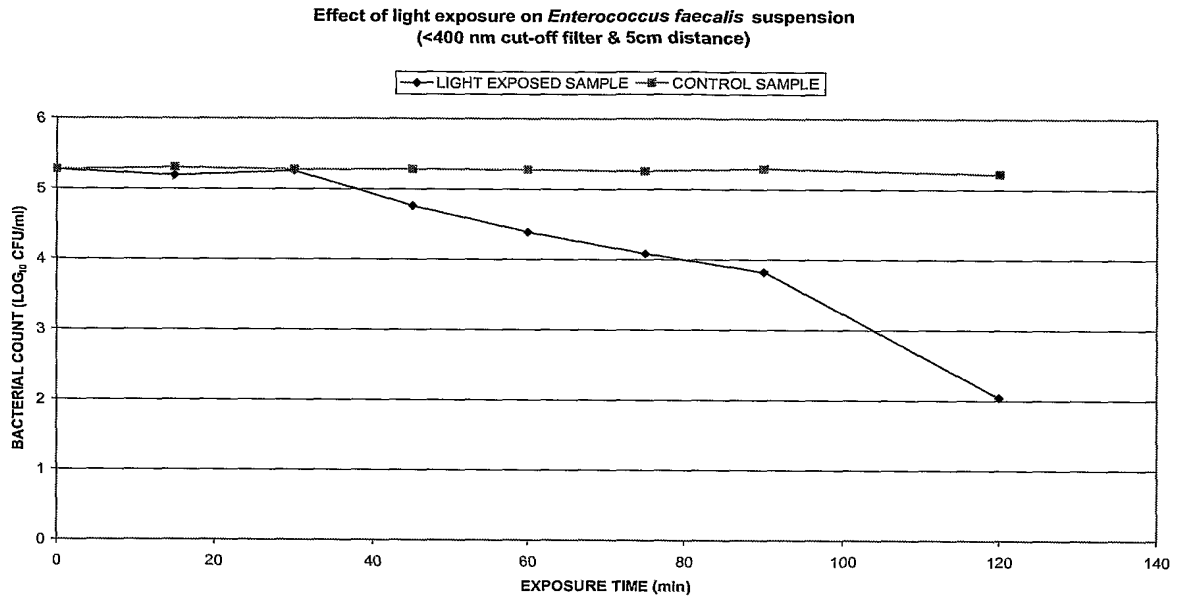
Effect of light exposure on *Enterococcus faecalis* suspension
(<400 nm cut-off filter & 5cm distance)
LIGHT EXPOSED SAMPLE
CONTROL SAMPLE
BACTERIAL COUNT (LOG_{10} CFU/ml)
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
EXPOSURE TIME (min)

FIGURE 8

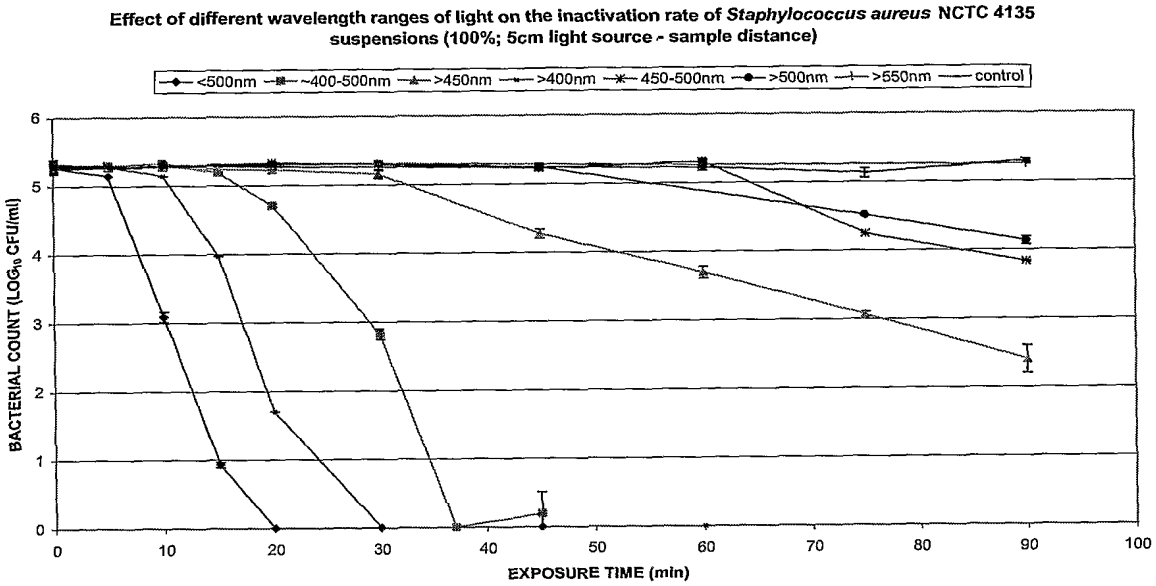
Effect of different wavelength ranges of light on the inactivation rate of Staphylococcus aureus NCTC 4135 suspensions (100%; 5cm light source - sample distance)
<500nm
~400-500nm
>450nm
>400nm
450-500nm
>500nm
>550nm
control
BACTERIAL COUNT (LOG10 CFU/ml)
EXPOSURE TIME (min)
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70
80
90
100

FIGURE 9

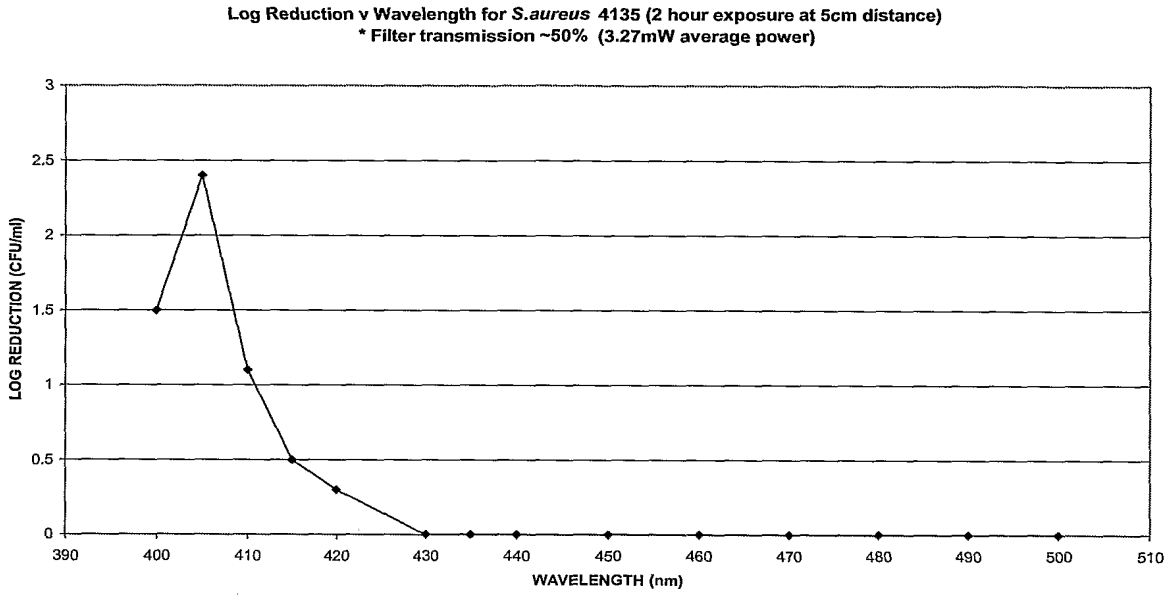
Log Reduction v Wavelength for S.aureus 4135 (2 hour exposure at 5cm distance)
* Filter transmission ~50% (3.27mW average power)
LOG REDUCTION (CFU/ml)
3
2.5
2
1.5
1
0.5
0
390
400
410
420
430
440
450
460
470
480
490
500
510
WAVELENGTH (nm)

FIGURE 10

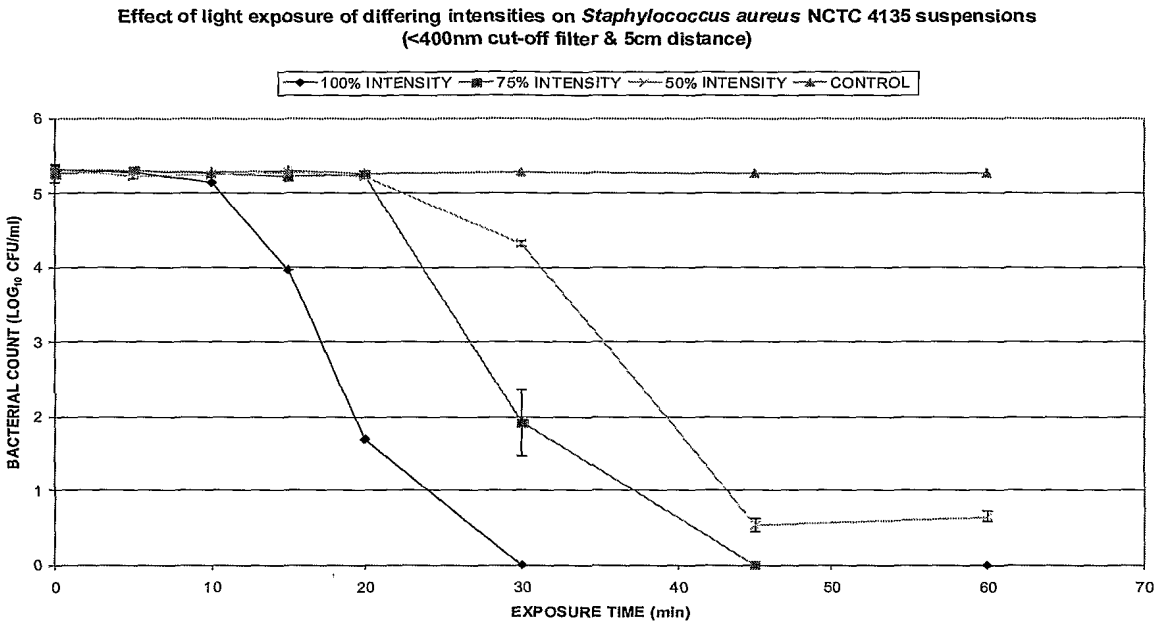
Effect of light exposure of differing intensities on *Staphylococcus aureus* NCTC 4135 suspensions
(<400nm cut-off filter & 5cm distance)
100% INTENSITY
75% INTENSITY
50% INTENSITY
CONTROL
BACTERIAL COUNT (LOG10 CFU/ml)
EXPOSURE TIME (min)
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70

FIGURE 11

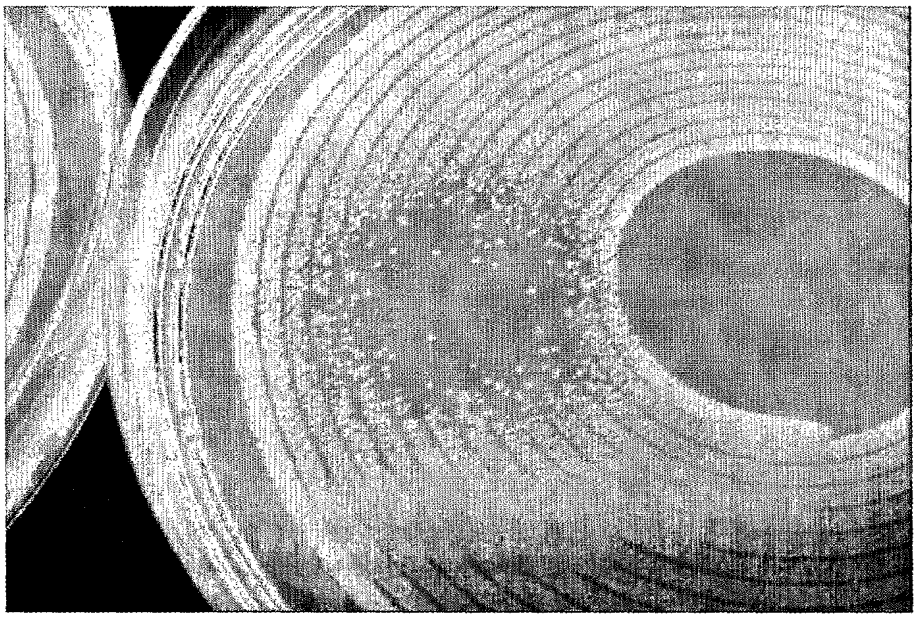

FIGURE 12

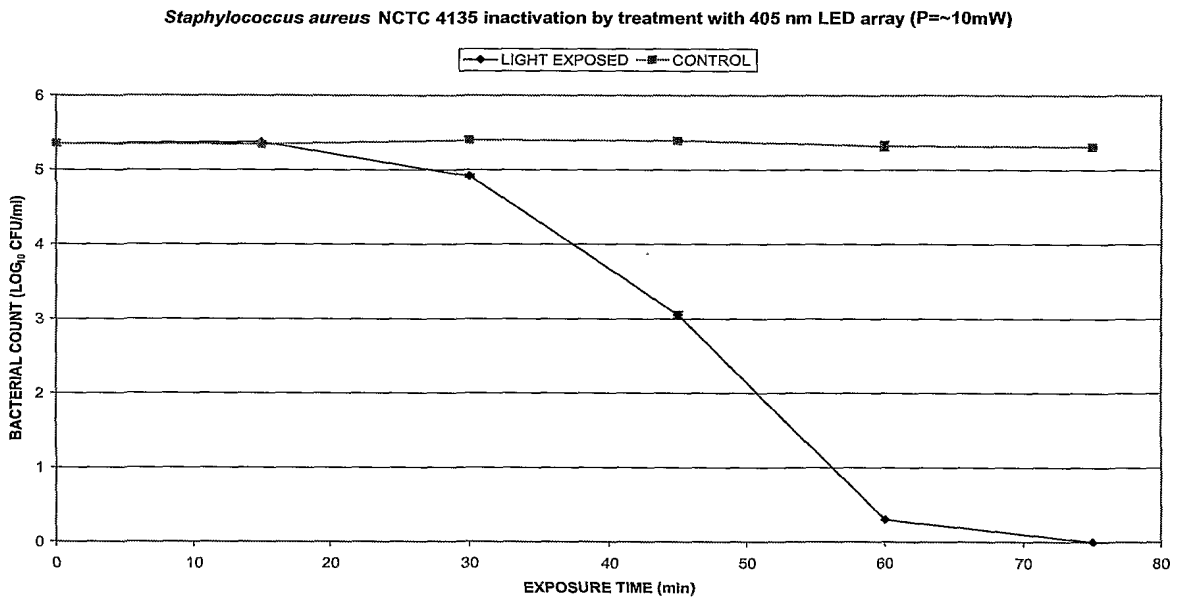
Staphylococcus aureus NCTC 4135 inactivation by treatment with 405 nm LED array (P=~10mW)
LIGHT EXPOSED
CONTROL
BACTERIAL COUNT (LOG_{10} CFU/ml)
EXPOSURE TIME (min)
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70
80

FIGURE 13

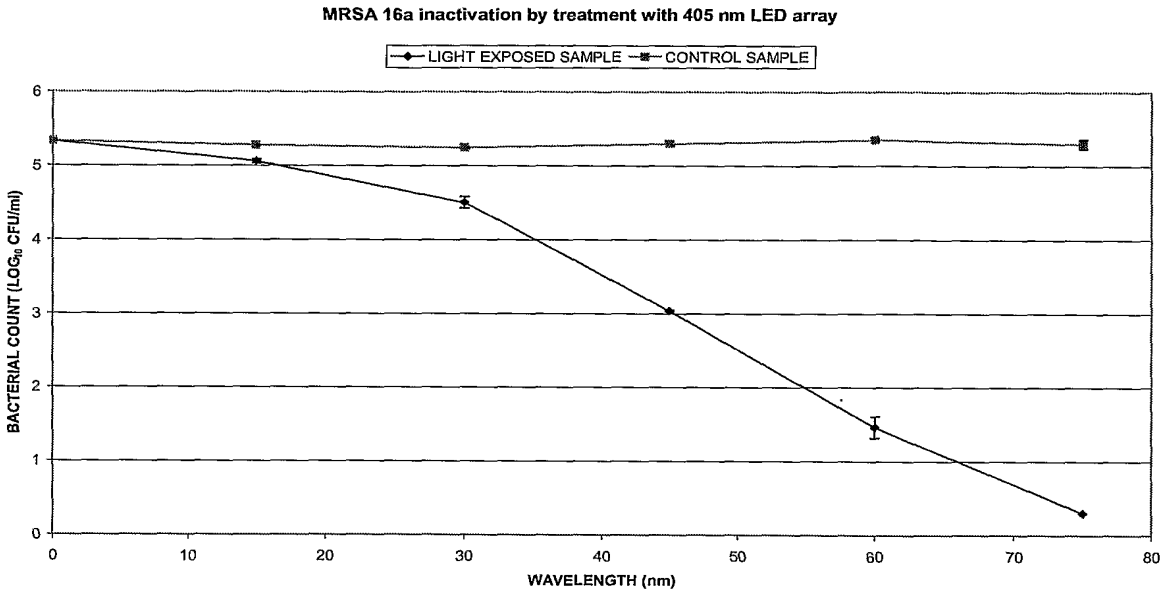
MRSA 16a inactivation by treatment with 405 nm LED array
LIGHT EXPOSED SAMPLE
CONTROL SAMPLE
BACTERIAL COUNT (LOG10 CFU/ml)
WAVELENGTH (nm)
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70
80

FIGURE 14

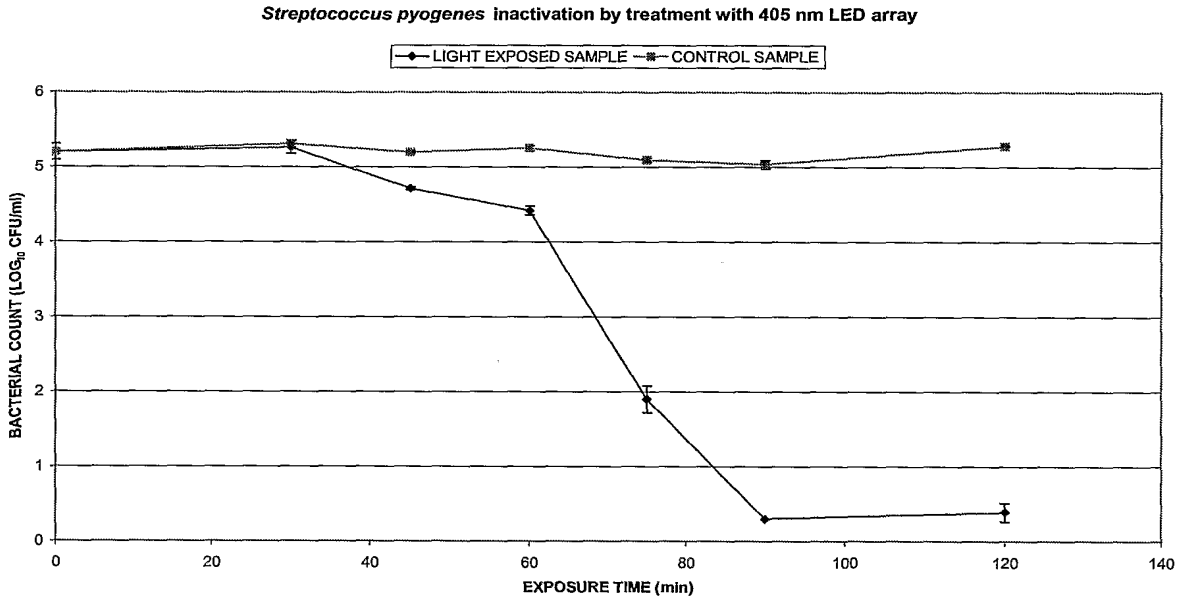
Streptococcus pyogenes inactivation by treatment with 405 nm LED array
LIGHT EXPOSED SAMPLE
CONTROL SAMPLE
BACTERIAL COUNT (LOG_{10} CFU/ml)
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
EXPOSURE TIME (min)

FIGURE 15

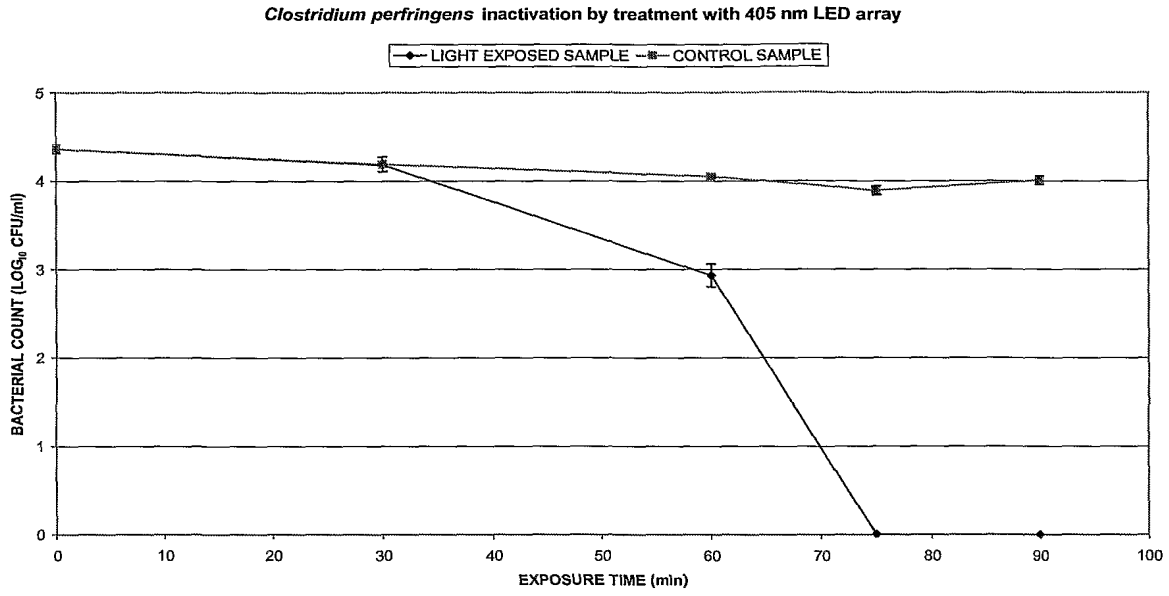
Clostridium perfringens inactivation by treatment with 405 nm LED array
LIGHT EXPOSED SAMPLE
CONTROL SAMPLE
BACTERIAL COUNT (LOG_{10} CFU/ml)
0
1
2
3
4
5
0
10
20
30
40
50
60
70
80
90
100
EXPOSURE TIME (min)

FIGURE 16

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2006/002841

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61N5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/055070 A1 (JONES GARETH [GB] ET AL) 10 March 2005 (2005-03-10) abstract; claim 15 paragraph [0070] - paragraph [0071]	12,13
X	US 2004/147986 A1 (BAUMGARDNER JONATHAN M [US] ET AL) 29 July 2004 (2004-07-29) abstract paragraph [0017] - paragraph [0018] paragraph [0085] - paragraph [0089]	12,13
X	WO 2005/048811 A2 (JAY HARVEY H [US]) 2 June 2005 (2005-06-02) abstract page 24, line 8 - page 25, line 7 page 37, line 11 - line 14	12,13

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 November 2006	17/11/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Beck, Ewa

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2006/002841

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-11,14,15
because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all *searchable claims.*

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2006/002841

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2005055070 A1	10-03-2005	NONE	
US 2004147986 A1	29-07-2004	WO 2004071409 A2	26-08-2004
WO 2005048811 A2	02-06-2005	US 2005045189 A1	03-03-2005
		US 2005049657 A1	03-03-2005